

Table 3
Referral source and treatment history (n = 106).

Characteristic		Note
Number of psychiatrists consulted by client before intake (%)		
Never consulted	15 (14.2)	
One psychiatrist	46 (43.4)	
Two psychiatrists	26 (24.5)	
Three or more psychiatrists	19 (17.9)	
Time from first help-seeking behavior to intake (mean ± SD, days)	449.3 ± 675.7	Median, 143; range, 0–2692
Time from first contact with a mental health professional to intake (mean ± SD, days)	388.8 ± 617.0	Median, 91; range, 0–2688
Referral source (%)		
Psychiatrist	72 (67.9)	
Private outpatient clinic	25	
Outpatient clinic at hospital	43	
Other mental health services	4	
School counselor/nursing teacher	10 (9.4)	
Non-psychiatric doctor	13 (12.3)	
Self-referral	11 (10.4)	
Past diagnosis by psychiatrist (%) ^a		
ARMS/suspected ARMS	15 (16.5)	15 have never met psychiatrist
Psychosis/suspected psychosis	7 (7.7)	
Schizophrenia/suspected schizophrenia	23 (25.3)	
Other psychiatric disorder	26 (28.5)	
Unknown/other	20 (22.0)	
Medication by previous psychiatrist (%)		
Antipsychotic ^b	39 (36.8)	
Typical	16 (15.1)	
Atypical	25 (23.6)	
Antidepressant	23 (21.7)	
Mood stabilizer	3 (2.8)	

^a Diagnosis primarily by referral. When referral was not from a psychiatrist, other past diagnoses were adopted, if available.

^b Two were prescribed both typical and atypical antipsychotics.

individuals with ARMS/suspected ARMS. Thirty-nine (36.8%) of the participants were taking antipsychotics at intake. Among the 72 individuals referred from psychiatrists, 29 (40.8%) were prescribed antipsychotics.

4. Discussion

The present study naturalistically followed up 106 people with ARMS for an average period of 2.4 years. This study's estimated transition rates of 7.5% at 6, 11.1% at 12, and 15.4% at 24 months were relatively lower than those reported in a previous meta-analysis (Fusar-Poli et al., 2012), in which transition rates at 6, 12, and 24 months were 17.7%, 21.7%, and 29.1%, respectively. However, they are consistent with other recently published transition rates; i.e., 15.2% at 24 months (Fusar-Poli et al., 2013b) and 15.6% at 24 months (Ziermans et al., 2011). Because many of our participants were help-seeking young individuals primarily referred from psychiatrists in a clinical setting, we tried to offer the best available interventions, psychosocial or pharmacological. Although we could not unambiguously attribute subjects' improvement to our interventions available evidence (Fusar-Poli et al., 2012; van der Gaag et al., 2013) suggests that these psychological and pharmacological interventions can reduce the risk of conversion. Therefore, there is a possibility that psychosis onset may be prevented or delayed in at least a subset of the ARMS subjects in this study, though further rigorous study is necessary to confirm this. We also need careful consideration about the use of antipsychotics, since evidence regarding a benefit of antipsychotics in these populations is still scarce, and antipsychotics are known to have various potentially strong adverse effects (Stafford et al., 2013).

The present findings support the importance of a phenomenological assessment of ARMS (Valmaggia et al., 2013). Although some authors questioned the significance of the APS in predicting conversion (Yung et al., 2005; Velthorst et al., 2009), many studies found more severe APS at baseline in converters compared to non-converters (Cannon et al., 2008; Ruhrmann et al., 2010; Liu et al., 2011; Nelson et al., 2013). In the present study, we assessed attenuated delusional symptoms with distinction of "unusual thought content" (i.e., attenuated bizarre ideas, in which ego-boundary disturbance symptoms were

included) and "non-bizarre symptoms" and found that converters had more severe scores and fulfilled APS item criteria more often in the "unusual thought content" item, but not in the "non-bizarre ideas" item. "Disorganized speech" also had a similar trend, but not perceptual abnormalities. Additionally, we found that "emotional disturbance," in which subjective emotional disturbance, observed blunted affect, and observed inappropriate affect are included, was more severe in converters than non-converters, implying that some features of negative symptoms could be useful for predicting conversion to psychosis (Đemjaha et al., 2012; Valmaggia et al., 2013). Intriguingly, we found that compared to non-converters, converters fulfilled more of the four APS items in the CAARMS, and 78.6% of the converters had more than three APS items. Furthermore, half of the individuals showing more than three APS items converted to psychosis. Because many diagnostic criteria for sub-threshold disorders in other medical conditions (e.g., dementia with Lewy bodies and polymyositis) adopt a strategy of counting the number of more specific symptoms and biological markers, it seems reasonable to apply a similar approach to ARMS diagnosis and encourage studies identifying more specific symptoms and biological markers with predictive value.

The baseline characteristics of the participants generally replicated previous studies (Cannon et al., 2008; Ruhrmann et al., 2010; Fusar-Poli et al., 2013b; Nelson et al., 2013). Notably, in our sample no one had a history of substance use, including cannabis. This is much lower than the proportion of substance use and comorbid diagnosis of substance abuse/dependence reported in studies from Western countries (Phillips et al., 2002; Addington et al., 2007; Ruhrmann et al., 2010; Fusar-Poli et al., 2013b). Furthermore, while several studies observed an association of a history of substance abuse with future development of psychosis (Kristensen and Cadenhead, 2007; Cannon et al., 2008), not all have (Phillips et al., 2002). The use of cannabis and/or other substances may have a causal relationship with schizophrenia or psychotic conditions (e.g., Rapp et al., 2012; Burns, 2013), so their impact on the presentation of ARMS and risk of conversion should be carefully considered.

The proportion of individual on antipsychotic medication appears to be markedly lower at the SAFE clinic than other specialized services in East Asia, whereas the proportion of individuals on antipsychotic

medication for ARMS seems relatively high [79.7% initially treated with antipsychotics in Taipei (Liu et al., 2011), 65.2% during the follow-up period in Seoul (Kwon et al., 2012), and 77.8% at the 1-year follow-up point in Tokyo (Morita et al., 2014)]. Our data are better reflective of the other leading centers in the world: 28% of the followed-up subjects with available data in the OASIS (Outreach and Support in South London) (Fusar-Poli et al., 2013b), 25.2% of the subjects with available information in the EPOS (European Prediction of Psychosis Study) (Ruhmann et al., 2010), and 35.1% during the follow-up interval in the NAPLS (North American Prodrome Longitudinal Study) (Cannon et al., 2008) were on antipsychotics. Many of our medicated participants had stopped antipsychotics at the end-point if they did not convert or were clinically stable. There was concern about inappropriate antipsychotic prescription in individuals with ARMS, and the present findings support the idea that ARMS could contribute to reducing inappropriate antipsychotic medication (Woods et al., 2010), and that antipsychotic medication might be necessary and useful only for a subset of ARMS individuals (Phillips et al., 2009; van der Gaag et al., 2013).

In the study, two-thirds of the participants were referred from psychiatrists and over 85% of them had been seen by at least one. This differs greatly from studies in other cities such as London and Toronto (Fusar-Poli et al., 2013b; Stowkowy et al., 2013), in which one-third of the referrals came from general practitioners (GPs) and the others from diverse sources including community mental health teams, early intervention services, and psychologists. In Japan, there are no certified GPs serving as primary care specialists, and Japanese primary care physicians are less likely to refer patients to psychiatric services (Fujisawa et al., 2007). In addition, many family members consult psychiatrists, rather than other physicians, before a patient's first visit (Hagi et al., 2013). Since people can consult a psychiatrist directly with walk-in access under universal health insurance coverage, dozens of patients visit outpatient psychiatric clinics and are seen by psychiatrists. Although easy access to psychiatric specialists is beneficial for many patients, most psychiatrists do not have sufficient time to see patients with complex presentations like ARMS. There is also a concern about overdiagnosis of schizophrenia and overprescription of antipsychotics in Japanese clinical settings (Mizuno et al., 2012). In our sample, one-third of the participants were diagnosed with or suspected to have psychosis or schizophrenia by previous psychiatrists, and 40% of the participants referred by psychiatrists had received antipsychotics. The rate of antipsychotic treatment might have been higher if psychiatrists had begun treatment before referring patients to our clinic. Improving education for psychiatrists and implementing an appropriate clinical service system is necessary in Japanese clinical settings to address this population's needs.

As is usual with longitudinal studies, one of the main limitations of the study is patient attrition: 25.4% of the subjects stopped visiting the clinic before the 1-year follow-up, and we could not follow up with 21.7% of the subjects. Although the only difference between the dropped-out and followed-up subjects was more severe subjective depressive symptoms in the former group, we cannot preclude the possibility that the more severe cases ceased treatment and developed psychosis thereafter. On the other hand, in our followed-up sample, severity of the depressive symptoms was not associated with conversion. This observation is consistent with the findings that comorbid diagnosis of anxiety and depressive disorders at baseline were not associated with transition to psychosis (Fusar-Poli et al., 2014).

This study has some more limitations. Firstly, the sample size was smaller than that of several larger multicenter studies (Cannon et al., 2008; Ruhmann et al., 2010), as well as that of well-funded leading centers (e.g., Fusar-Poli et al., 2013b; Nelson et al., 2013); the small sample size made it difficult to conduct a multivariate analysis to identify risk factors that had contributed to developing psychosis. Secondly, the follow-up period of 2.4 years, on average, may not be long enough to obtain the true conversion rate of our sample, since the risk of developing psychosis is known to increase over time (Fusar-Poli et al.,

2013a), including at up to 10 years after initial identification (Nelson et al., 2013). Thirdly, the transition rate and other outcomes may have been affected by not controlling the intervention and by sample bias, since this was a naturalistic study. Fourthly, we did not use a specific instrument to assess depression in psychosis, such as the Calgary Depression Scale (CDS) (Addington et al., 1993), which could have assessed depression more precisely in the present population. Additionally, although we assessed functional outcome using GAF, as was done in previous studies (e.g., Ruhmann et al., 2010; Nelson et al., 2013), a more detailed assessment of function using a specialized rating scale such as GF: social and role (Cornblatt et al., 2007) would be beneficial to evaluate the functional outcome of ARMS. Finally, because ours was a university hospital clinic with sufficient work force to provide individuals with careful attention, it is unclear whether the findings can be generalized to Japanese ordinal clinical settings.

This is one of the largest naturalistic longitudinal studies of an ARMS population conducted in non-Western regions. Despite several differences in the referral sources and clinical sample characteristics, the transition rate to psychosis was in line with that recently seen elsewhere in the world. Our results emphasize the importance of the phenomenological assessment of ARMS, especially the assessment of ego-boundary symptoms, formal thought disorder, and emotional disturbance, to predict development of psychosis. A specialized clinic for ARMS may have a pivotal role in educating clinicians and implementing the best available service for ARMS in a clinical setting, in which potential overdiagnosis of schizophrenia and overprescription of antipsychotics cause concern.

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Contributors

K.M. and M.K. designed the study and wrote the manuscript. K.M. and H.M. contributed to managing the project. M.K., N.O., and C.O. managed the data and M.K. analyzed them. K.M., N.O., M.K., T.M., F.L., C.O., and T.K. recruited, clinically evaluated, and followed up the participants, and offered them treatment. They also approved the final manuscript.

Conflicts of interest

All authors declare no conflicts of interest for the work presented here.

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Suicidal Feelings Interferes with Help-Seeking in Bullied Adolescents

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Abstract

Purpose: Being bullied is associated with the manifestation of suicidal feelings, which sharply increase in middle(-late) adolescence. Whether or not bullied middle(-late) adolescents with suicidal feelings seek help is therefore a critical issue, given that help-seeking plays a key role in the prevention of suicide. The aim of the present study is to investigate the effects of bullying, suicidal feelings and the interaction between these two factors on help-seeking behavior in adolescents.

Methods: Japanese middle(-late) adolescents (aged 15–18 years; $n = 9484$) were studied using self-report questionnaires. The rate of adolescents who actually sought help was examined for bullying status and suicidal feelings.

Results: The rate of adolescents who sought help was significantly higher when they were bullied ($p < 0.001$) and also when they had mild suicidal feelings ($p < 0.001$), but not when they displayed serious suicidal feelings. In the case of adolescents who were bullied, however, having suicidal feelings significantly decreased the rate of help-seeking ($OR = 0.47$, $p < 0.05$ and $OR = 0.32$, $p = 0.002$ for having mild and serious suicidal feelings, respectively). The decrease was remarkable when suicidal feelings were serious. Specifically, the decrease was significant in seeking help from peers and family members, who are the most frequent source of the help for adolescents, when they had serious suicidal feelings ($OR = 0.21$, $p < 0.01$ and $OR = 0.13$, $p < 0.001$, respectively).

Conclusions: Suicidal feelings may interfere with help-seeking behavior, which could be critical in suicide prevention in bullied middle(-late) adolescents.

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Data Availability: The authors confirm that, for approved reasons, some access restrictions apply to the data underlying the findings. This study was planned and conducted in accordance with the ethics committee of the Tokyo Metropolitan Institute of Psychiatry. When applying to the research ethics committee for our data set, we did not request this to be released as public data. However, the data can be made available to all interested researchers upon request to Atsushi Nishida Ph.D.

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Introduction

Suicide is a leading cause of death in adolescence. The rate of suicide increases dramatically during the teenage years [1–3]. Worldwide, the total number of suicides among people in early adolescence (aged 10–14 years) in 2004 was 11,000 (the tenth leading cause of death), but this jumped to 60,000 (the second leading cause of death) among people in middle(-late) adolescence (aged 15–19 years) [2]. In developed countries, suicide is the second leading cause of death in those aged 15–19 years, following traffic accidents as the first leading cause of death. [2] In 2011, in Japan, the rate of suicide (per 10,000 people) in early adolescents

was 1.3, but this increased to 8.5 in middle(-late) adolescents [4]. Thus, prevention of suicide, especially in these adolescents, is one of the most significant issues in adolescent mental health [2,3,5,6].

While suicide and suicidal behaviors are generally multifactorial [3,5–7], bullying and help-seeking may play significant roles in these behaviors and their prevention in adolescents [3,6,8–13]. In adolescents, seeking help from non-health professionals, including peers, family members and teachers, and not only from professionals, is thought to play a major role in the prevention of suicide. Adolescents are more likely to seek help from non-professionals than professionals, and these non-professionals can

serve as a pathway to professional services which can help prevent suicide [5,12,14].

Bullying is extremely prevalent in children and adolescents. A large-scale school-based epidemiological study observed that 30% of children in the U.S. (Grades 6–10th: 11–15 years) were involved in bullying, including as pure victims (those who are victimized and do not bully others; 11%), pure bullies (those who bully others and are not victimized themselves; 13%) and bully-victims (those who both bully and are victimized themselves; 6%) [15]. A school-based survey across 40 countries, including a number of European countries, Turkey, Israel and North American countries, found that 8.6–45.2% of children (11–15 years) were involved in bullying [16]. Several cross-sectional school-based studies observed that being bullied may be associated with the risk of suicidal behaviors in Finland, Korea and the U.S. [10,17–20]. Longitudinal population-based cohort studies observed the association between victimization by bullying in childhood or adolescence and later mental disorders and suicidal behavior, including completed suicide, in the U.K. and Finland [21–24]. However, help-seeking behavior in bullied adolescents generally still remains to be studied. While some school-based studies in the U.K. have reported that bullied adolescents may be hesitant to consult others [25–27], studies in the U.K., Norway and the U.S. have observed that they may seek more help when bullied [28–30].

The relation between suicidal feelings and help-seeking is another important issue, which remains to be further studied. A U.S. epidemiological study found that two to three times more adolescents with suicidal feelings sought help, including formal help-seeking from health care professionals, than those without these feelings [31]. Australian and U.S. studies that investigated the intention to seek help, not actual behavior, however, observed a decreased intention in most adolescents with suicidal feelings [32,33].

The present study therefore investigated the effects of bullying, suicidal feelings and their interaction on help-seeking behaviors in middle(-late) adolescents (aged 15–18 years). We hypothesized that suicidal feelings interfere with help-seeking in adolescents who are victims of bullying. The rate of adolescents who actually sought help was studied by bullying status and suicidal feelings using a large school-based sample. Help-seeking behaviors directed at different sources, including peers and family members (or informal help-seeking), not only health-care professionals (or formal help-seeking), were examined, given the fact that, as mentioned above, help from such sources is thought to play a role in reducing the risk of suicide [12,14].

Method

Subjects

A cross-sectional survey of psychopathologies was conducted between 2008 and 2009 in Kochi prefecture (population: 790,000), Japan. Data were collected from students at 28 senior high schools (10th–12th grades, aged 15–18 years). Out of 9,991 students at 28 senior high schools, 256 students (2.6%) were absent on the days of the survey, and 251 students (2.5%) did not agree to participate in this study. Thus, a total of 9,484 students (95.3% of 9,991, 4546 males and 4938 females, age: 16.6±0.93 years (mean ±s.d.), range = 15–18 years) answered the questionnaire.

For the practical procedure of the study, the principal investigators approached the school principals and asked them to co-operate with the survey, and the principals then consulted with teachers and parents. After we had obtained written informed consent from the parents, the teachers handed a self-report questionnaire and envelope to each student. When they were distributed, the teachers explained to the students that: 1) participation in the study was anonymous and voluntary, and 2) strict confidentiality would be maintained. The students were requested to seal the completed questionnaire in the envelope provided. Research staff collected the sealed questionnaires at each school.

The study was planned and conducted in accordance with the Ethical Guidelines for Epidemiological Research of Japan, and approved by the ethics committees of the Tokyo Metropolitan Institute of Psychiatry, the Mie University School of Medicine, and the Kochi Medical School, Kochi University.

Measures

The questionnaire included items on: 1) bullying and victimization, 2) suicidal feelings, 3) actual help-seeking behaviors, and 4) other variables including demographic characteristics such as sex and age. An expert in child and adolescent psychology and three teachers from the participating schools, including a Japanese language teacher, examined the questions for age-appropriateness and reading difficulty.

Bullying status. Experiences of being bullied or bullying others were assessed using the following questions: 1) “Have you been bullied within the past year?” and 2) “Have you bullied others within the past year?” with a choice of two responses, “Yes” or “No.” Based on the responses, we classified the subjects into the following four groups: 1) those who did not bully and were not victimized (uninvolved); 2) those who bullied others and were not

Table 1. Frequencies of suicidal feelings by bullying status in middle(-late) adolescents.

Adolescents	Suicidal feelings				
	(n = 9431)	Mild	(n = 815)	Serious	(n = 434)
	n	n	(%)	N	(%)
Bullying status ^b					
Uninvolved	8657	706	(8.2)	324	(3.7)
Pure bullies ^d	372	42	(11.3)	42	(11.3)
Pure victims ^c	295	50	(16.9)	48	(16.3)
Bully-victims ^c	107	17	(15.9)	20	(18.7)

Note. Suicidal feelings are significantly associated with bullying status in middle(-late) adolescents (^b $p < 0.001$, Kruskal-Wallis test). Severity of suicidal feelings is significantly higher in those bullied (pure victims and bully-victims) than in the uninvolved and pure bullies (^c $p < 0.001$, Bonferroni post-hoc test), and the severity in pure bullies is significantly higher than in the uninvolved (^d $p < 0.001$, Bonferroni post-hoc test) in middle(-late) adolescents. (The total number of subjects is less than the number of subjects analyzed, due to missing data for suicidal feelings or bullying status.)

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Table 2. The number of the adolescents who sought help for psychological distress according to bullying status^a and sources of help.

	Total of subjects 3161	(n = 8407) 37.6%	Uninvolved 2844	(n = 7734) 36.8%	Pure bullies 131	(n = 318) 41.2%	Pure victims 142	(n = 261) 54.4%	Bully-victims 44	(n = 94) 46.8%
Sources of help*										
Informal help-seeking	3003		2715		148		144		48	
Peers	2771		2492		127		115		37	
Family members	1192		1064		43		66		19	
Teachers	134		102		10		15		7	
Formal help-seeking (Health care professionals)	222		169		16		25		12	

Note: The number of students who sought help, divided according to the total number of adolescents, is shown by bullying status. The frequencies of the subjects who sought help are significantly associated with bullying status in the adolescents ($p < 0.001$, Kruskal-Wallis test). The frequency is significantly higher among pure victims than among the uninvolved and pure bullies ($p < 0.001$, with Bonferroni test). *Multiple answers. doi:10.1371/journal.pone.0106031.t002

victimized themselves (pure bullies); 3) those who were victimized and did not bully others (pure victims); and 4) those who both bullied others and were victimized themselves (bully-victims).

Suicidal feelings. Suicidal feelings were measured using the following question: "Do you currently have thoughts that your life is no longer worth living?" [34] The participants selected one of four possible responses: "No", "Probably not", "Possibly yes", or "Yes." Based on the responses, we classified the subjects into the following three groups: 1) responses of "No" and "Probably not" were regarded as the absence of suicidal feelings (none), 2) responses of "Possibly yes" were regarded as the presence of mild suicidal feelings (mild), and 3) responses of "Yes" were regarded as the presence of serious suicidal feelings (serious).

Help-seeking behavior and resources for help. Actual help-seeking behaviors regarding psychological stresses were measured using the following question: "Have you recently consulted anyone to discuss your psychological stress or problems?" Participants selected one of two possible responses: 1) "No, I am not consulting anybody", or 2) "Yes, I am currently consulting (or seeking help from) somebody about my stress or psychological problems." The former was defined as "not seeking help", and the latter as "seeking help". If they selected 2) "yes", they were asked to specify whom they were consulting or seeking help from. This question was answered by selecting one or more of the following: peers, family members, teachers, school nurses, psychologists, doctors or others.

Statistical analysis

Associations between bullying status and suicidal feelings and between bullying status and the rate of adolescents who actually sought help were analyzed using the Kruskal-Wallis test, with post hoc comparison using the Bonferroni test.

The association between suicidal feelings and the rate of adolescents who actually sought help was examined by bullying status using logistic regression. The odds ratio for help-seeking in adolescents with mild or serious suicidal feelings was compared to those without suicidal feelings according to each bullying status. Sex and age were controlled for in the analysis. All statistical analyses were conducted using the Statistical Package for Social Science (SPSS), version 21.0 for Macintosh (IBM Inc., New York, U.S.).

Results

Frequencies of suicide feelings and bullying

The frequencies of suicidal feelings and bullying and their relations in the middle(-late) adolescents are summarized in Table 1. The frequencies of adolescents with mild and serious suicidal feelings were 815 and 434 out of 9,431 (8.6% and 4.6%) in the adolescents. Among these, the frequencies of pure bullies, pure victims and bully-victims were 4.0%, 3.1% and 1.1%.

Suicidal feelings were significantly associated with bullying status in the adolescents ($\chi^2 = 260.30$, $d.f. = 3$, $p < 0.001$, Kruskal-Wallis test). Suicidal feelings were significantly higher among those who were bullied (pure victims and bully-victims) than among the uninvolved and pure bullies ($p < 0.001$ in Bonferroni post-hoc test), and were significantly higher among pure bullies than among the uninvolved adolescents ($p < 0.001$ in Bonferroni post-hoc test).

Frequencies of help-seeking by bullying status

As summarized in Table 2, the rate of adolescents who sought help was 37.6%. The Severity of suicidal feelings was significantly associated with the bullying status ($\chi^2 = 38.96$, $d.f. = 3$, $p < 0.001$, Kruskal-Wallis test). The frequency was significantly higher

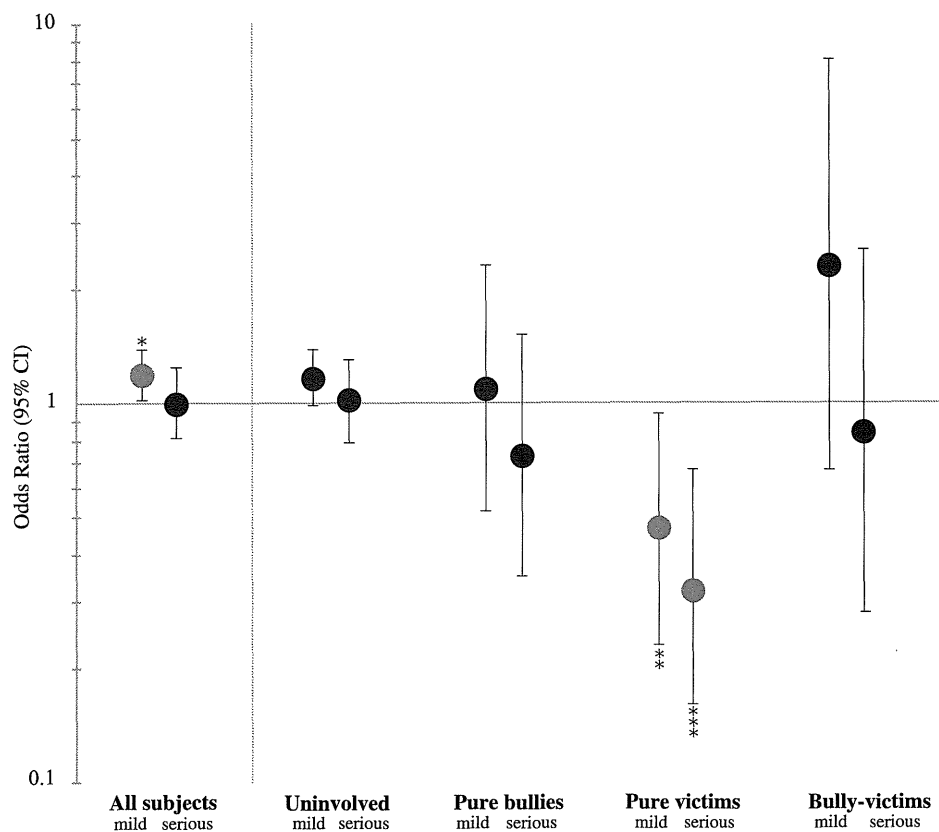


Figure 1. Interactive effects of suicidal feelings and bullying status on help-seeking for psychological distress in middle(-late) adolescents. Note. Odds ratio for seeking help (adjusted for gender and age); 95% CI=95% confidence interval; mild = having mild suicidal feelings; serious = having serious suicidal feelings. Reference = having no suicidal feelings. In each section, subjects with missing data were excluded from the statistical analyses. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. doi:10.1371/journal.pone.0106031.g001

among pure victims (54.4%) than the uninvolved (36.8%) and pure bullies (41.2%) ($p < 0.001$, with Bonferroni test).

Interactive effects of suicidal feelings and bullying status on help-seeking

The interactive effects of suicidal feelings and bullying status on help-seeking in the adolescents are summarized in Figure 1. In the total group of subjects, the rate of adolescents who sought help was significantly higher when they had mild suicidal feelings than when they did not have suicidal feelings (OR = 1.19, 95% CI: 1.02–1.39, $p < 0.05$). However, a striking finding was that the rate was significantly reduced in pure victims among the adolescents when they had suicidal feelings compared with when they did not have suicidal feelings (OR = 0.47, 95% CI: 0.23–0.94, $p < 0.05$ for mild suicidal feelings, and OR = 0.32, 95% CI: 0.16–0.67, $p = 0.002$ for serious suicidal feelings, respectively). This effect of suicidal feelings in the bullied adolescents was due to the fact that there was no increase in help-seeking, which was higher in the bullied adolescents without suicidal feelings, as summarized in Table 3. As shown in Table 2, the pure victims mostly sought informal help (144 of the 261 pure victims) when they sought any help. Among the informal help-seeking, the most frequent source of help was peers ($n = 115$), followed by family members ($n = 66$), while a few sought help from school teachers ($n = 15$). The number of pure victims who sought formal help was also low (29 out of 261). As summarized in Figure 2, seeking help from peers and family members was significantly reduced in the victims with serious

suicidal feelings (OR = 0.21, 95% CI: 0.09–0.51, $p < 0.01$ and OR = 0.13, 95% CI: 0.04–0.45, $p < 0.001$, respectively), compared to those without suicidal feelings.

Discussion

The present study first investigated the effects of bullying status and suicidal feelings on actual help-seeking behavior in middle(-late) adolescents (aged 15–18 years). Cross-sectional data from a large school-based sample were analyzed. The rate of adolescents who actually sought help was significantly higher in the bullied adolescents compared with the uninvolved and bullying adolescents. What was more striking, however, was that the higher level of help-seeking behavior in the bullied (or pure victims) disappeared when they had suicidal feelings. This interaction between being bullied and having suicidal feelings was more noticeable when the suicidal feelings were serious, rather than mild. The effect was more noticeable when suicidal feelings were serious. Hence, the present study suggests that in bullied middle(-late) adolescents, having suicidal feelings may obstruct the increase in help-seeking behavior. The present finding may have a significant impact on the prevention of suicidal behaviors, because middle-late adolescence is an age when the risk of suicide sharply increases[2,35] and help-seeking behavior may be of great significance in the prevention of suicide at this age. In addition, suicidal feelings were more frequent in the victims of bullying than in the uninvolved and in bullies, consistent with previous studies[10,17–21]. To the best of our knowledge, the present

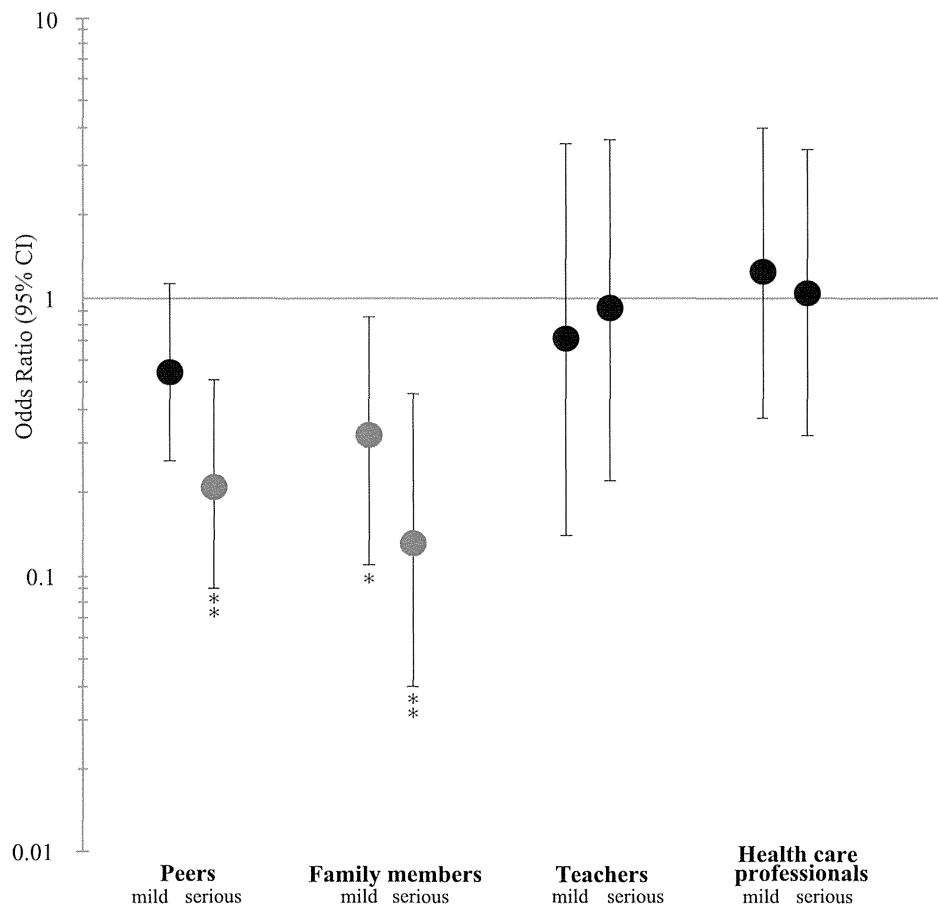


Figure 2. Effects of suicidal feelings on help-seeking for psychological distress by source of the help, in pure victims. Note. Odds ratio for seeking help from the source (adjusted for gender and age); 95% CI=95% confidence interval; mild = having mild suicidal feelings; serious = having serious suicidal feelings. Reference = having no suicidal feelings. *: $p < 0.05$; **: $p < 0.01$. doi:10.1371/journal.pone.0106031.g002

study is the first to observe the change in help-seeking in bullied adolescents with suicidal feelings.

Except for the middle(-late) adolescents with suicidal feelings, help-seeking behavior was higher in bullied adolescents. The relation between bullying and help-seeking has not been clarified. Several studies have reported that bullied children and adolescents may hesitate to consult others [25,26]. Other studies, however, have observed that they may seek more help when bullied [28–30]. The present results are consistent with the latter studies, but further research is required to draw a definite conclusion.

Regarding the relation between suicidal feelings and help-seeking behavior, the rate of adolescents who sought help was higher when they had mild suicidal feelings. This elevation was not observed when suicidal feelings were serious. A previous epidemiological study found that significantly more adolescents with suicidal feelings sought help than those without such feelings [31]. Studies investigating the intention to seek help, not actual behavior, however, have made contrasting findings [32,33,35,36]. The present results are partly consistent with the previous studies that investigated actual behavior. A higher rate of help-seeking behavior was, however, not observed in the adolescents with serious suicidal feelings in the present study.

The most frequent sources of help in the bullied middle(-late) adolescents were peers and family members. The help-seeking behavior directed toward these informal sources was significantly reduced when the bullied middle(-late) adolescents had suicidal

feelings. In contrast, formal help-seeking (e.g., from school nurses and health care professionals) was rare among the pure victims, regardless of the presence or absence of suicidal feelings. The decrease in help-seeking (mostly informal help-seeking) in those with suicidal feelings may be related to the previous observations of elevated isolation in adolescents with suicidal feelings [37,38]. Isolation is a major factor in committing suicide [5,39,40]. The decreased help-seeking from peers and family members in those with suicidal feelings may enhance the isolation at school and at home, which could block the channel of seeking help at the time of great risk of committing suicide.

The following limitations must be acknowledged. First, this is a cross-sectional survey, and therefore the causal relationships among suicidal feelings, bullying and help-seeking are not clear. Second, the participants were asked about help-seeking for “psychological stress or problems” in the questionnaire. Help-seeking for suicidal feelings or bullying was not asked about directly. This could affect the observed associations between suicidal feelings, bullying and help-seeking. Third, we did not ask the participants about the detailed features of bullying, including frequency, duration or type (e.g., physical, verbal, relational, or cyber bullying). Bullying-type specific relations between suicidal feelings and help-seeking remain to be further studied. Fourth, we used a simple sentence to ask about suicidal feelings. Therefore, we were not able in the present study to discriminate between non-imminent suicidal feelings and imminent severe suicidal ideas

Table 3. The rate of middle(-late) adolescents who sought help for psychological distress, according to bullying status and suicidal ideation.

	Total of subjects		Uninvolved		Pure bullies		Pure victims		Bully-victims	
Total	3161/8407	(37.6)	2844/7734	(36.8)	131/318	(41.2)	142/261	(54.4)	44/94	(46.8)
Suicidal feelings										
None ^a	2701/7300	(37.0)	2470/6823	(36.2)	99/245	(40.4)	104/170	(61.2) ^b	28/62	(45.2)
Mild	314/725	(43.3)	266/629	(42.3)	17/35	(48.6)	22/47	(46.8)	9/14	(64.3)
Serious	146/382	(38.2)	108/282	(38.3)	15/38	(39.5)	16/44	(36.4)	7/18	(38.9)

Note. The number of subjects who sought help, divided by the total number of adolescents, is shown by bullying status and suicidal feelings, with the rate (%) in brackets. Without suicidal feelings, the rate of the subjects who sought help was significantly associated with bullying status in middle adolescents ($p < 0.001$, Kruskal-Wallis test), while the rate in pure victims was significantly higher than the uninvolved and pure bullies ($p < 0.001$, Bonferroni post hoc test). With suicidal feelings, however, the rate did not increase among victims of bullying. (The total number of subjects is less than the number of subjects analyzed due to missing data for suicidal feelings and bullying status.)

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which lead to real suicide attempts. The effect of having such imminent, suicidal ideas should be further studied. Fifth, the present findings could be specific to the Japanese middle(-late) adolescents.

The present study suggests that in bullied middle(-late) adolescents, having serious suicidal feelings may not increase help-seeking behaviors. This finding is critically important, because this means that help-seeking behavior may not be increased in the adolescents in the most need of the help. Health care professionals and school staff should be aware of this finding

and may need to have appropriate skills to inquire about suicidal feelings or ideas in adolescents, especially in those being bullied.

Author Contributions

Conceived and designed the experiments: SS YO. Performed the experiments: AN TS SS. Analyzed the data: YK FT TS. Contributed reagents/materials/analysis tools: YK. Contributed to the writing of the manuscript: YK TS. All guidance: TS.

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RESEARCH ARTICLE

Hippocampal Subfield Volumes in First Episode and Chronic Schizophrenia

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Abstract

Background

Reduced hippocampal volume in schizophrenia is a well-replicated finding. New imaging techniques allow delineation of hippocampal subfield volumes. Studies including predominantly chronic patients demonstrate differences between subfields in sensitivity to illness, and in associations with clinical features. We carried out a cross-sectional and longitudinal study of first episode, sub-chronic, and chronic patients, using an imaging strategy that allows for the assessment of multiple hippocampal subfields.

Methods

Hippocampal subfield volumes were measured in 34 patients with schizophrenia (19 first episode, 6 sub-chronic, 9 chronic) and 15 healthy comparison participants. A subset of 10 first episode and 12 healthy participants were rescanned after six months.

Results

Total left hippocampal volume was smaller in sub-chronic ($p = 0.04$, effect size 1.12) and chronic ($p = 0.009$, effect size 1.42) patients compared with healthy volunteers. The CA2-3 subfield volume of chronic patients was significantly decreased ($p = 0.009$, effect size 1.42) compared to healthy volunteers. The CA4-DG volume was significantly reduced in all three patient groups compared to healthy group (all $p < 0.005$). The two affected subfield volumes were inversely correlated with severity of negative symptoms ($p < 0.05$). There was a small, but statistically significant decline in left CA4-DG volume over the first six months of illness ($p = 0.01$).



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Conclusions

Imaging strategies defining the subfields of the hippocampus may be informative in linking symptoms and structural abnormalities, and in understanding more about progression during the early phases of illness in schizophrenia.

Introduction

Reduced hippocampal volumes are a more prominent morphological feature of chronic schizophrenia in comparison to the more subtle volumetric changes seen in first episode or early schizophrenia patient groups [1–3]. The hippocampus consists of major subfields, including the Cornu Ammonis (CA)1, CA2-3, dentate gyrus (DG), presubiculum, and subiculum. The perforant pathway input from the entorhinal cortex induces serial excitatory transmission through DG, CA2-3, CA1, and back to the entorhinal cortex through the subiculum. This trisynaptic pathway has been considered to be the fundamental network closely linked to learning and memory [2]. Several papers in hippocampal morphology in schizophrenia have indicated that pathological alterations were evident in subfield or hemisphere specific manner [4–6]. Although the significance of structural changes in hippocampal subfields was proposed, it remained uncertain whether the subfields would be globally reduced or specific parts of subfields such as CA1, CA3, DG or subiculum are subject to more localized change.

Recently, more detailed analyses of hippocampal shape and surface morphometry have revealed both localized and lateralized findings [7–11], including subregional differences into CA1 and CA2 subfields in first episode patients [9]. A multimodal imaging strategy indicated that deformations in the anterior hippocampus were related to cortical thinning, and the degree of disruption in structural hippocampal-cortical connectivity was associated with severity of negative symptoms [10]. Progressive changes in hippocampal shape were also reported [12]. Using a three-dimensional surface mapping technique, the extent of “deflation” of the hippocampus in schizophrenia was associated with duration of illness in a sample of 67 patients with mean age 39 years and duration of illness 13 years [13]. Severity of both positive and negative symptoms was also associated with this measure of change in hippocampal structure. The finding of an association between surface shape abnormalities referable to the CA1 subfield and positive symptoms was also observed in a study of patients mean age 34 and duration of illness 10 years [14].

Current high-resolution image segmentation strategies now provide both the opportunity for surface mapping, as well as the opportunity to obtain hippocampal subfields volumes from magnetic resonance images [15]. These newer imaging strategies allow for the assessment of subfields including CA4 and the dentate gyrus that may not be readily detected by surface analysis techniques. Recently, a large study including chronic schizophrenia, schizoaffective disorder, and bipolar disorder with psychosis reported smaller total hippocampal volume across all diagnostic groups compared to healthy volunteers, with the most prominent reductions seen in the schizophrenia group within the CA2-3, CA4-DG and the subicular subfields [16]. In this study smaller volumes were associated with more severe positive symptoms, and greater cognitive impairment, but not with antipsychotic treatment. A second study of hippocampal subfield volumes in 21 multi-episode chronic patients reported an association between CA1 and CA2-3 volumes and positive symptoms [17]. Negative symptoms were not associated with subfield volumes in this study, and comparisons to subfield volumes in healthy participants was not

reported. Finally, a study of young family members of probands with schizophrenia reported smaller subiculum volumes compared with healthy controls with no family history [18].

Studies of first episode patients, with minimal medication treatment, and longitudinal studies are required to determine if specific subfields are affected early in the course of illness, and if progressive changes occur. Similar strategies to investigate cognitive impairment in the elderly have suggested that initial volume loss in CA1 was associated with mild cognitive impairment, followed by volume loss across multiple subfields and transition to Alzheimer's disease [19]. While cognitive deficits are an established characteristic of schizophrenia, whether the relationships between hippocampal subfield and cognitive deficits are similar in schizophrenia compared to Alzheimer's patients are unknown.

For the current investigation we hypothesized that the volumes of hippocampal subfields CA1, CA2-3, DG, presubiculum, or subiculum would be smaller in chronic schizophrenia compared with healthy participants, and that there would progressively larger differences related to duration of illness. Furthermore, we expected the degree of volume change to be correlated with the severity of psychopathology. We used a high-resolution segmentation strategy based on a recently developed automated technique (see [15]) to examine hippocampal subfields in groups of first episode, sub-chronic and chronic patients in comparison with healthy participants. As well, to further examine the possibility of progression, we performed a second scan six months following baseline in a subset of first episode patients and healthy comparison participants.

Methods

Participants

Thirty-four ethnically Japanese patients were recruited from in- or outpatient services of Kochi Medical School, Hosogi Unity Hospital, and Tosa Hospital in Kochi Prefecture, Japan. Demographic data and clinical features of illness appear in Table 1. Nineteen of the patients were in their first episode of illness at baseline, defined as having made their first contact for treatment of psychotic symptoms, and having less than one month of lifetime antipsychotic treatment. Patients who were ill for six months to five years were categorized as sub-chronic ($N = 6$), and who were ill for over five years as chronic ($N = 9$). Exclusion criteria included a history of head injury with loss of consciousness, other neurological disorder, current substance abuse or dependence. Participants had no clinically significant brain pathology, as determined by a neuro-radiologist's review of the MRI scans. The diagnosis of schizophrenia was made according to ICD-10 criteria. Socio-economic status (SES) was analyzed based on educational years [20]. Clinical assessments included the Positive and Negative Syndrome Scale (PANSS) [21], the Clinical Global Impression (CGI) [22], and Global Assessment of Functioning (GAF) (DSM-IV-TR). The Schizophrenia Cognition Rating Scale (SCoRS), an 18 item scale of real world cognitive deficits, was used to evaluate cognitive functioning. The global rating of SCoRS was analyzed in retrograde fashion [23,24]. Chlorpromazine dose equivalents were calculated for all antipsychotic drugs [25–27]. First episode, sub-chronic and chronic patients were scanned at baseline. Ten first episode patients were rescanned after six months (mean 192 days, SD 16). First episode patients were taking stable doses of antipsychotic medication (risperidone $n = 6$, olanzapine $n = 3$, perospirone $n = 1$; mean chlorpromazine equivalents = 300 mg) at the time of the follow-up scan. Fifteen healthy volunteers were recruited from the neighboring region. Healthy controls had no past or current history of mental illness, and met the same exclusion criteria as patients. Twelve healthy volunteers were rescanned after six months (mean 186 days, SD 15).

Table 1. Demographic and clinical characteristics of participants at baseline, mean (standard deviation).

	Healthy Controls		Schizophrenia					
	(N = 15)		FES (N = 19)		SCS (N = 6)		CS (N = 9)	
Age (yrs) ^a	25.0	(4.6)	25.1	(6.8)	22.2	(3.7)	36.8	(6.7)
SES ^b	5.7	(0.9)	4.5	(1.2)	4.2	(1.6)	4.0	(1.0)
Parental SES	4.9	(1.0)	4.6	(1.1)	4.3	(0.8)	4.3	(1.2)
SCoRS			4.0	(1.3)	5.2	(2.3)	5.4	(1.8)
Handedness (right, left)	15, 0		15, 4		5, 1		9, 0	
Sex (M, F)	10, 5		9, 10		3, 3		6, 3	
Chlorpromazine-Equivalent dose at scan (mg/day) ^c			263	(156)	758	(218)	781	(116)
PANSS score								
Positive subscale			16.8	(3.1)	16.7	(6.1)	19	(5.1)
Negative subscale ^d			14.6	(4.4)	19	(4.9)	18.1	(2.8)
General subscale			30.1	(5.4)	32.7	(4.9)	35.8	(6.1)
Total score			61.5	(10.3)	68.3	(14.7)	72.9	(12.0)
CGI			4.3	(0.7)	4.2	(1.2)	4.4	(0.9)
GAF			45.8	(9.5)	50	(16.7)	44.8	(12.7)

SES: socio-economic status, SCoRS: Schizophrenia cognition rating scale, PANSS: Positive and Negative Syndrome Scale, CGI: Clinical global impression, GAF: Global assessment of function, FES: first episode schizophrenia, SCS: sub-chronic schizophrenia, CS: chronic schizophrenia

^a Overall difference between groups $F = 10.9, p < 0.001$

^b Overall difference between groups $F = 5.36, p = 0.003$

^c Different between groups $F = 11.9, p < 0.001$

^d Different between groups $F = 3.78, p = 0.03$

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Ethical considerations

Ethical review and approval for this study was provided by the Kochi Medical School ethical committee. Subjects were recruited from two streams of independent participants. For subjects enrolled in our stand-alone longitudinal study all subjects directly provided written informed consent for our specific study. Additional subjects ascertained from part of a separate cross-sectional study provided broad-based informed written consent to have their medical records and charts included in any institution-based research projects, such as the current study. Those whose data were included from the cross-sectional pool were given notification of the inclusion of their medical records via the medical school's webpage interface, as stipulated by the clinical ethics committee. In cases where the participants may have had a compromised capacity or ability to give consent, next of kin, care takers or guardians consented on their behalf.

Image acquisition and processing

All participants were examined on 1.5 T GE scanner at Kochi Medical School using a 3D T1-weighted SPGR sequence (TE = 4 ms; TR = 9.7 ms; flip angle = 12°; matrix = 256 × 256; FOV = 200 mm; slice thickness = 1.5 mm with no gap; 0.78 × 0.78 × 1.5 mm voxels; 124 slices). Total intracranial and hippocampal volumes were determined using the FreeSurfer software package version 5.1 (<http://surfer.nmr.mgh.harvard.edu/fswiki>). The hippocampus segmentation was fully automated without manual editing. All automated outputs were visually inspected to ensure there were no technical failures or mislabeling. Two subjects were excluded after visual

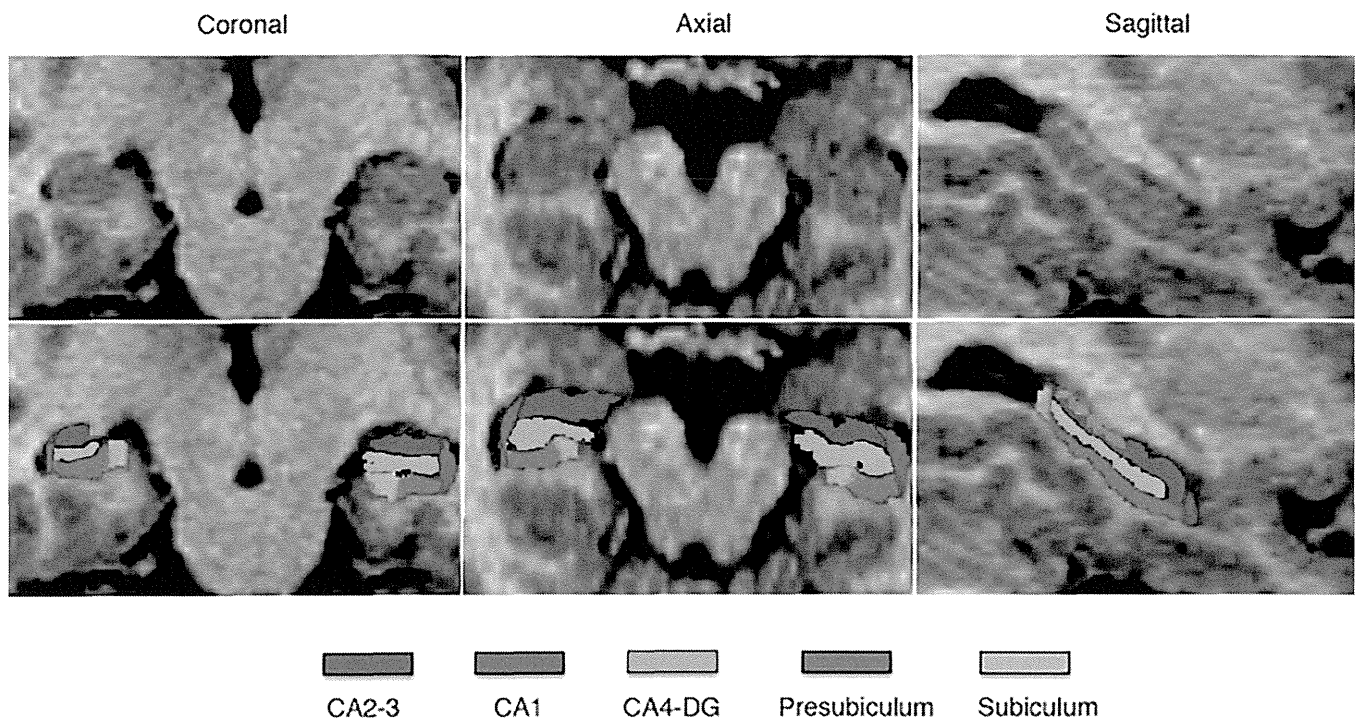


Fig 1. Hippocampal subfield segmentation in a representative subject.

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examination due to failure of the segmentation algorithm. The FreeSurfer definition of the hippocampal subfields includes the dentate gyrus (CA4-DG), the Ammon’s horn subfields (CA1, CA2-3), the subiculum and presubiculum [15]. The hippocampal subfield delineations are illustrated in Fig. 1. The delineated images were similar to the previous results processed with FreeSurfer [15,17,19,28]. The mean subfield volumes we obtained are very similar to those reported by the algorithm developers [15]. As well, we calculated the intraclass correlation coefficient for $n = 12$ scans of healthy participants performed six months apart. The ICC for the left hippocampus total volume was 0.90, and for the right 0.86. For the 10 left and right hippocampal subfields, the mean ICC value was 0.88, with a range of 0.71 for the right CA1 subfield, to 0.95 for the left CA2-3. For longitudinal analyses, we used FreeSurfer’s longitudinal stream to process two serial MRIs from study participants to provide accurate estimates of subtle changes over time [29].

Statistical analyses

To test the main hypotheses concerning total hippocampal and subfield volumes at baseline, analysis of variance (ANOVA) was performed with group (first episode schizophrenia, sub- or chronic schizophrenia, control) entered as a main effect, and age and intracranial volume entered as covariates. Whole left and right hippocampal volumes were tested first, with a p -value set at 0.025 for statistical significance. A difference in total volume between groups was followed up by subsequent statistical comparisons of the five subfield volumes, with a p -value set at 0.01 for statistical significance. For post-hoc comparisons of total or subfield volumes between the three diagnostic groups, t -tests were used with the Hochberg approach to correct for multiple testing, as previously used in other MRI studies [16,30,31]. Repeated measures ANOVA was performed on hippocampal subfield volume in the first episode group and in healthy volunteers for those who had follow-up imaging with group as a between-subjects factor, and time (baseline, follow-up) as a within-subjects repeated measure.

Demographic and clinical variables were analyzed with chi-square or ANOVA as appropriate. Relationships between hippocampal total and subfield volumes, demographic and clinical assessments (duration of illness, age, positive and negative symptoms) were explored with parametric or non-parametric techniques depending on the distribution properties of the variables. Analyses were conducted using SPSS version 22.0 (SPSS Tokyo, Japan) or JMP 10 (SAS Institute, Cary NC, USA).

Results

Demographic and clinical data of participants

Demographic and clinical variables in the cross-sectional study are presented in Table 1. ANOVA of the four groups revealed significant differences in age ($F = 10.9$, $p = 0.036$). Post hoc analyses revealed that the mean age of chronic group was significantly older than other groups. Although there were significant differences in scores of SES between the groups ($F = 5.4$, $p = 0.003$), we did not find differences in parental SES. Chlorpromazine-equivalent doses at scan in sub-chronic and chronic group were higher than first episode group. Scores for negative symptom severity were higher in the sub-chronic and chronic groups than in the first-episode group. SCoRS, CGI, and GAF did not show significant differences between groups. In the longitudinal study, the first episode took a mean dose of 290 mg chlorpromazine equivalent antipsychotics for about 15 days before the first scan. Controls had higher SES score than first episode group, but there was no difference in parental SES between the groups. There was no difference in age, sex, or intracranial volume between the groups. First episode subjects at follow-up were significantly improved in total score ($F = 5.65$, $p = 0.041$) and positive symptom score ($F = 9.41$, $p = 0.013$) in PANSS, CGI ($F = 21.00$, $p = 0.001$), and GAF ($F = 17.80$, $p = 0.002$).

Hippocampal total and subfield volumes at baseline

Total hippocampal volume on the left was significantly different between groups, with both healthy and first episode schizophrenia participant volumes larger than chronic schizophrenia (Table 2). In sub-chronic schizophrenia the mean volume of the left hippocampus was 12.6% smaller than controls ($p = 0.04$, effect size 1.12); in chronic schizophrenia, the mean volume was 17.0% smaller than controls ($p = 0.009$, effect size 1.42) (Fig. 2).

The hippocampal subfields showed distinctive patterns of differences between groups. Volumes of the presubiculum and CA1 subfields were relatively unchanged, and although the subiculum was smaller in chronic patients, there was no statistically significant difference between groups. In contrast, the CA2-3 region was smaller in sub-chronic ($p = 0.03$, effect size 1.19) and chronic patients ($p = 0.006$, effect size 1.50) than healthy volunteers, with first episode patients having intermediate volumes that were not different from either group. The volume of the CA4-DG subfield was significantly smaller in first episode ($p = 0.004$, effect size 1.06), sub-chronic ($p = 0.004$, effect size 1.51) and chronic groups ($p = 0.0003$, effect size 1.89) compared to healthy volunteers.

Associations with clinical features of illness

There were no statistically significant correlations between age and total hippocampal or age and subfield volumes on either side. We conducted exploratory analyses of associations between duration of illness and volumes, using Kendall's tau as the measure since the data was not normally distributed, and because there were multiple zero values for duration of illness when the first episode participants were included. As seen in Fig. 2, statistically significant

Table 2. Total and subfield volumes (ml) for the hippocampus at baseline.

	Healthy (N = 15)		FES (N = 19)		SCS (N = 6)		CS (N = 9)		Healthy vs FES		Healthy vs SCS		Healthy vs CS	
	LSM	(SEM)							p-value	Cohen d	p-value	Cohen d	p-value	Cohen d
Left hippocampus ^a	4270	(111)	4078	(92)	3800	(166)	3607	(169)	0.19	0.46	0.04	1.12	0.009	1.42
CA1	345	(10)	316	(8)	319	(15)	305	(16)						
CA2-3 ^b	1000	(26)	921	(22)	883	(39)	834	(40)	0.03	0.8	0.03	1.19	0.006	1.5
CA4-DG ^c	571	(14)	517	(11)	491	(21)	460	(21)	0.004	1.06	0.004	1.51	0.0003	1.89
Presubiculum	466	(16)	468	(13)	461	(24)	454	(24)						
Subiculum	630	(20)	606	(16)	581	(30)	543	(30)						
Right hippocampus	3943	(116)	4118	(96)	4048	(174)	3997	(176)						
CA1	333	(10)	326	(8)	351	(15)	345	(15)						
CA2-3	922	(26)	941	(21)	971	(39)	941	(39)						
CA4-DG	522	(15)	525	(12)	542	(22)	533	(23)						
Presubiculum	481	(17)	470	(14)	437	(25)	432	(25)						
Subiculum	618	(18)	606	(15)	598	(27)	599	(28)						

Hippocampal volumes are corrected for age, sex and intracranial volume. LSM: least squares mean, SEM: standard error of the mean. Post-hoc p-values are corrected for three comparisons. FES: first episode schizophrenia, SCS: sub-chronic schizophrenia, CS: chronic schizophrenia

^aDifference between groups $F = 4.51, p = 0.008$
^bDifference between groups $F = 4.87, p = 0.005$
^cDifference between groups $F = 8.14, p = 0.0002$

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associations were observed for total left hippocampus (Kendall's tau = -0.38, $p = 0.004$), as well as left CA2-3 (Kendall's tau = -0.39, $p = 0.003$) and CA4-DG (Kendall's tau = -0.40, $p = 0.002$). These findings must be interpreted cautiously, as removal of the first episode participants decreased the strength of the association below the level of statistical significance.

Severity of positive symptoms did not correlate with the three target hippocampal subfield volumes. Severity of negative symptoms was correlated with left CA2-3 ($r^2 = 0.16, p = 0.02$) and with left CA4-DG ($r^2 = 0.21, p = 0.007$), but not with total left hippocampal volume (Fig. 2). Effects of negative symptom severity remained statistically significant when age, sex, and intracranial volume were added to the model. There were no statistically significant correlations between hippocampal volumes and antipsychotic doses.

Changes in hippocampal total and subfield volumes over the first six months of treatment

At follow-up, the severity of total and positive symptoms was reduced in the first episode schizophrenia patients (see Table 3). Total hippocampal volumes did not differ over time between healthy volunteers and patients with first episode schizophrenia. The left CA4-dentate gyrus subfield showed a statistically significant group-by-time interaction ($p = 0.01$). Volumes increased slightly in healthy volunteers over six months, and declined slightly in first episode schizophrenia (Fig. 3 and Table 4).

Discussion

In this study, the CA2-3 and CA4-DG subfields of the left hippocampus were more prominently reduced than the CA1 region, or the presubiculum/subiculum in patients with schizophrenia. The graded differences in subfield volumes between health volunteers, first episode, and

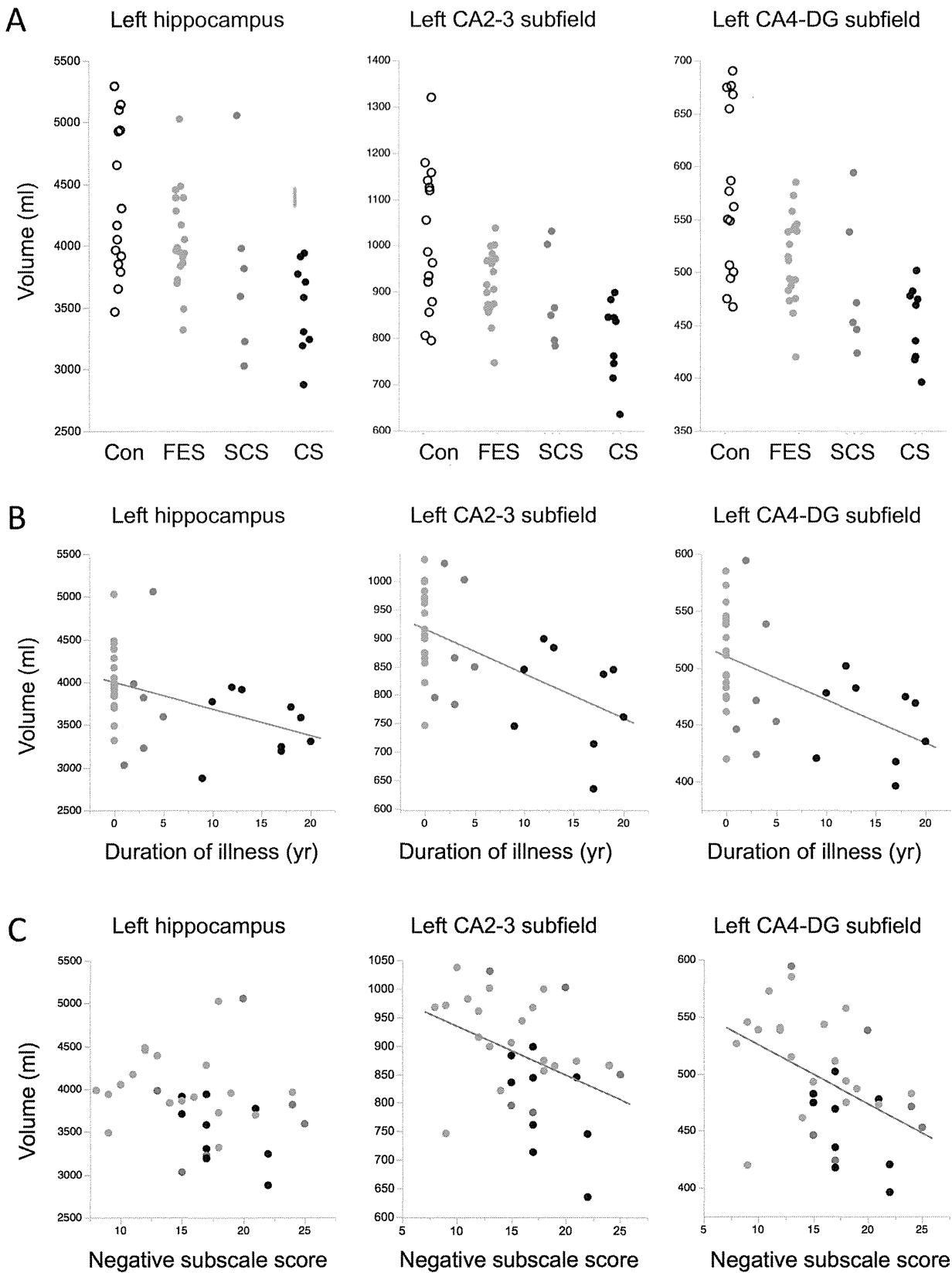


Fig 2. Left hippocampal total and subfield volumes in control and schizophrenia groups. (A) Hippocampal total and subfield volumes in healthy controls (Con, black open circles), first episode schizophrenia (FES, blue), sub-chronic schizophrenia (SCS, red) and chronic schizophrenia (CS, black) participants. Mean volumes were smaller in CS than Con for total ($p = 0.004$), CA2-3 ($p = 0.003$) and CA4-dentate (DG) ($p < 0.001$) subfields. **(B)** Relationships between duration of illness and hippocampal volumes. Non-parametric correlations were statistically significant for total ($p = 0.004$), CA2-3 ($p = 0.003$) and CA4-DG ($p = 0.002$). **(C)** Relationships between negative subscale scores on the PANSS and hippocampal volumes. Parametric correlations were statistically significant for CA2-3 ($p = 0.02$) and CA4-DG ($p = 0.007$).

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chronic schizophrenia, and the observation of increasing loss of volume in the CA4-DG subfield during the first six months following presentation for care, suggest that a dynamic process related to illness or treatment occurring. In the overall patient group more severe negative symptoms were associated with smaller subfield volumes.

The affected hippocampal subfields in this patient group are similar to those reported in postmortem studies to have neurochemical and synaptic changes associated with schizophrenia [2,4–6,32,33]. The relative predominance of findings on the left side, and the sparing of the CA1 region was also consistent with postmortem studies of the hippocampus in schizophrenia [5]. This is in contrast to the pathology of Alzheimer’s disease, where the CA1 region is affected early in the course of illness.

Of interest, abnormalities of the left hippocampus are more frequently observed schizophrenia [5]. Moreover, traumatic stress or abusive events in childhood have a propensity to contribute to smaller left hippocampal volumes in adulthood [34–37]. In addition to the preferential involvement of left hippocampus in schizophrenia, the asymmetric hippocampal changes in

Table 3. Demographic and clinical characteristics of participants in the longitudinal study, mean (standard deviation).

	Healthy Controls		First episode schizophrenia			
	(N = 12)		(N = 10)			
			Baseline		Six months	
Age (yrs)	25.4	(4.8)	25.8	(5.6)		
SES ^a	5.7	(0.7)	4.7	(1.3)		
Parental SES	4.5	(0.9)	4.3	(1.2)		
SCoRS	3.5	(1.1)	3.5	(0.9)		
Handedness (right,left) ^b	(12, 0)		(8, 2)			
Sex (M, F)	(5, 7)		(4, 6)			
Chlorpromazine-Equivalent dose at scan (mg/day)			290.0	(192)	310.0	(145)
PANSS score						
Positive subscale ^c			17.3	(2.8)	11.5	(5.6)
Negative subscale			13.6	(5.0)	11.9	(2.5)
General subscale			30.8	(5.7)	26.2	(7.4)
Total score ^c			61.7	(11.1)	49.6	(13.6)
CGI ^d			4.3	(0.8)	2.9	(1.1)
GAF ^d			43.3	(9.7)	63.8	(16.7)

Antipsychotics used were: risperidone (N = 6), olanzapine (N = 3), perospirone (N = 1). SES: socio-economic status, SCoRS: Schizophrenia Cognition Rating Scale, PANSS: Positive and Negative Syndrome Scale, CGI: Clinical global impression, GAF: Global assessment of function.

^a Overall difference between groups $F = 4.90, p = 0.04$

^b Different between groups Chi-square = 4.17, $p < 0.05$

^c Different over time, $F = 5.65–9.41, p < 0.05$

^d Different over time, $F = 17.80–21.00, p < 0.005$

doi:10.1371/journal.pone.0117785.t003

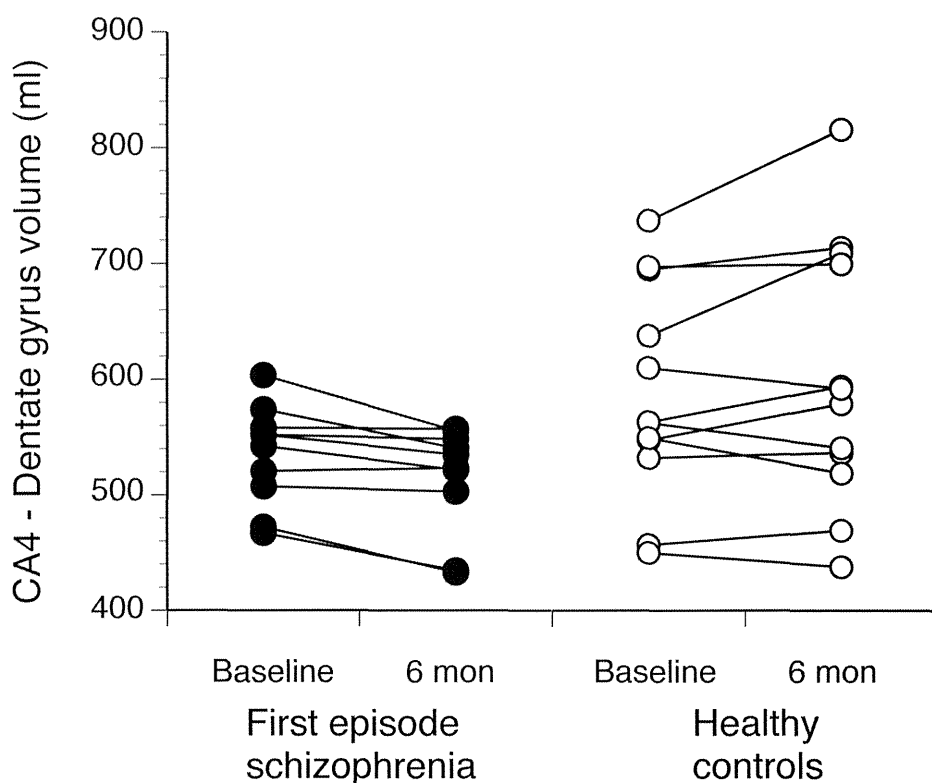


Fig 3. Baseline and follow-up volumes of left hippocampus CA4-dentate gyrus subfield in first episode schizophrenia and healthy control participants. A statistically significant diagnosis-by-time interaction was observed ($p = 0.01$).

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Table 4. Total and subfield volumes (ml) of participants in the longitudinal study, mean (standard deviation).

	Healthy Controls				First episode schizophrenia			
	(N = 12)				(N = 10)			
	Baseline		Six months		Baseline		Six months	
Left-Hippocampus	4481	(673)	4513	(722)	4248	(450)	4146	(556)
CA1	339	(56)	346	(58)	324	(25)	311	(29)
CA2-CA3	1036	(181)	1044	(186)	936	(77)	911	(89)
CA4-DG ^a	587	(92)	601	(113)	535	(43)	515	(46)
Presubiculum	483	(74)	479	(71)	458	(49)	456	(47)
Subiculum ^b	639	(106)	663	(111)	604	(43)	595	(52)
Right-Hippocampus	4291	(444)	4455	(706)	4138	(343)	4107	(379)
CA1	344	(54)	357	(64)	313	(23)	312	(20)
CA2-CA3	1014	(178)	1003	(150)	906	(70)	911	(54)
CA4-DG	574	(100)	575	(96)	508	(36)	512	(30)
Presubiculum	511	(85)	490	(63)	460	(53)	462	(61)
Subiculum	654	(85)	665	(89)	608	(45)	597	(49)

^a Group x time interaction $F = 7.62, p = 0.01$

^b Group x time interaction $F = 4.76, p = 0.04$

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